

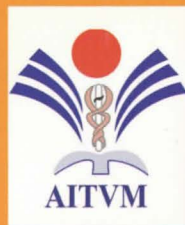
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Does control
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HIGH THROUGHPUT DIAGNOSIS OF AVIAN INFLUENZA: APPLICATION TO SURVEILLANCE OF WILD BIRDS

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ABSTRACT

In the context of highly pathogenic avian influenza (HPAI) emergence in Africa in early 2006, it has become necessary to increase the surveillance level in this region, including surveillance of wild birds suspected to play a role in transmission. A first surveillance campaign was launched in Africa in early 2006, within the framework of a regional Technical Cooperation Programme (TCP) of FAO. It showed a prevalence of 3.3% of LPAI in wild birds out of 5288 samples analysed manually. Here, we present our strategy for the development of a high throughput diagnostic line of AIV. Field collection of samples was carried out by Wetlands International and CIRAD. Cloacal and tracheal swabs collected from wild birds were immediately placed in conservative medium and sent to Montpellier where an automation workstation (Biomek FxP, Beckman) was used for RNA extraction. Specific quantitative RT-PCR were then run to identify influenza A virus positive samples and H5 or H7 subtypes. These subtypes could be further characterized by sequencing of the hemagglutinin cleavage site and by virus isolation. This strategy was successfully applied to wild bird surveillance in Africa and Eastern Europe during winter 2006/2007, within the framework of a second regional TCP of FAO.

INTRODUCTION

Influenza A viruses have been isolated from many species, including humans, pigs, horses, mink, felids, marine mammals and a wide range of domestic birds, but wildfowl and shorebirds are thought to form the virus reservoir in nature. Influenza A viruses are classified on the basis of two external proteins; the hemagglutinin (HA) and the neuraminidase (NA). In wild birds and poultry, viruses representing 16 HA and 9 NA subtypes have been detected. The HA glycoprotein is first synthesized as a HA0

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polypeptide precursor that is cleaved in HA1 and HA2 by proteases. Multiple basic amino acids at the cleavage site of this precursor are an absolute requirement for HPAI viruses resulting in a systemic lethal disease. Until now only influenza viruses of subtypes H5 and H7 have been associated with HPAI outbreaks in poultry.

The HPAI H5N1 epidemic that first appeared in China in 1996 has spread to Europe, Middle East and Africa in early 2006. In order to evaluate the role of wild birds in HPAI spread, a surveillance campaign was launched in Africa, within the framework of a regional Technical Cooperation Programme (TCP) of FAO and has shown a prevalence of 3.3% of LPAI in wild birds out of 5288 samples analysed by hand (Gaidet et al., 2007). Here, we present our strategy for the development of a high throughput diagnostic line of AIV applied to wild bird surveillance in Africa and Eastern Europe during winter 2006/2007, within the framework of a second regional TCP of FAO.

MATERIAL AND METHODS

Field collection of samples was carried out by Wetlands International and CIRAD. Cloacal and tracheal swabs collected from wild birds were immediately placed in conservative medium recommended by the World organisation for animal health (OIE) and sent to CIRAD using dry ice.

RNA extraction was carried out in an automation workstation (Biomek FxP, Beckman) using Nucleospin Multi 96 virus kit (Macherey Nalgen) in a biosafety level 3 laboratory.

Specific real-time quantitative RT-PCR were then run on a Stratagene Mx3000P to identify influenza A virus positive samples and H5 or H7 subtypes. Virulence was determined by nucleotide sequencing of the hemagglutinin cleavage site following specific RT-PCR.

RESULTS

At the time of writing the summary, the analyses were still in process. Around 6,900 samples from 9 countries out of 13,000 expected from 16 countries have been tested so far. No high pathogen viruses have been detected.

DISCUSSION

The high throughput diagnosis of AIV from tracheal and cloacal swabs has been developed in the laboratory. It is based on the use of an automation workstation for nucleic acid preparation on which a commercial kit (Macherey Nagel) gave us the best output compared to 4 others. Samples were distributed in a 96-well plate format and their RNA was extracted in 50 minutes without problems of contamination. The real-time RT-PCR adopted were those recommended by the FAO/OIE reference laboratory IZSve (Padova) and the Community reference laboratory VLA (Weybridge). The whole process including sample preparation, RNA extraction and real-time PCR for the M gene allowed to test up to 400 samples a day.

Acknowledgements

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